Appendices

Appendix 1.

Raw data and statistical analysis of the levels of protease produced by the *prtAΔ* strains, MK189 and MK190, and the wildtype strains, MH2 and MK191, under sulphur-nutrient-limiting conditions.

Table A1.1. Raw data. (arbitrary units protease activity/gram dry weight mycelia)

Strains		**MH2**		**MK191**		**MK189**		**MK190**	
conditions		**+S**	**-S**	**+S**	**-S**	**+S**	**-S**	**+S**	**-S**
Assay	1.1	3.76	140.43	0.95	161.61	4.42	17.18	3.51	16.25
	1.2	1.10	136,26	2.74	133.80	2.93	13.62	1.55	17.17
	2.1	7.72	218.35	0	196.8	3.29	28.79	5.91	4.89
	2.2	4.56	170.54	5.97	296	4.78	27.59	2.28	4.61
	3.1	5.90	170	6.02	172	5.80	16.26	2.36	14.62
	3.2	4.08	148	0.86	185.18	5.14	14.7	0.16	26.88
	mean	**4.52**	**163.93**	**2.75**	**190.90**	**4.40**	**19.69**	**2.63**	**14.07**
	SD	±2.22	±30.38	±2.66	±55.84	±1.10	±6.71	±1.95	±8.40

N.B. Assays were performed in triplicate, with internal duplicates. Assay numbering refers to assay number: duplicate number. Conidia was inoculated directly into sulphur-repressing medium (+S = sulphur-free minimal media (made with sulphur-free salt solution) containing 1% glucose as the carbon source, 10 mM ammonium tartrate as the nitrogen source, 0.1% thiosulphate as the sulphur source), or sulphur-derepressing medium (-S = sulphur-free minimal media (made with sulphur-free salt solution) containing 1% glucose as the carbon source, 10 mM ammonium tartrate as the nitrogen source, no sulphur source added). Cultures were grown for 20 hours.

Under conditions which repress extracellular protease production (+S), all four strains produced very low levels of protease activity. Under these conditions the assay results were very similar between all four strains.

The *prtAΔ* strains, MK189 and MK190, produce much lower levels of protease than the wildtype strains, under sulphur-nutrient limiting conditions (-S). The analysis of variance function ANOVA of Excel (Microsoft) was used to determine if the differences, observed in the assays examining protease production under sulphur-nutrient-limiting conditions, were statistically significant.

ANOVA: single factor, comparing MH2 and MK191 (the wildtype strains).

SUMMARY

Groups	Count	Sum	Average	Variance
MH2	6	983.57	163.93	922.82
MK191	6	1145.39	190.90	3117.78

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	2182.14	1	2182.14	**1.08**	0.323	**4.96**
Within groups	20203.01	10	2020.30			
Total	22385.16	11				

The F value of 1.08 is lower than the critical F value of 4.96, indicating that there is no significant difference between the MH2 and MK191 strains.

ANOVA: single factor, comparing MK189 and MK190 (the *prtAΔ* strains).

SUMMARY

Groups	Count	Sum	Average	Variance
MK189	6	118.13	19.69	44.99
MK190	6	84.41	14.07	70.52

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	94.76	1	94.76	**1.64**	0.23	**4.96**
Within groups	577.53	10	57.75			
Total	672.28	11				

The F value of 1.64 is lower than the critical F value of 4.96, indicating that there is no significance between the MK189 and MK190 strains.

As there was a no significant difference between the two wildtype strains, the data from these two strains can be combined. No significant difference exists between the two *prtAΔ* strains, so the data from these two strains can be combined. The combined wildtype and combined *prtAΔ* groups can be compared directly.

ANOVA: single factor, comparing wildtype and *prtAΔ* strains.

SUMMARY

Groups	Count	Sum	Average	Variance
wildtype	12	2128.96	177.41	2035.01
prtA.Δ	12	202.54	16.88	61.12

ANOVA

Source of variation	SS	df	MS	**F**	P-value	**F crit**
Between groups	154628.44	1	154628.44	**147.54**	3.15×10^{-5}	**4.3**
Within groups	23057.44	22	1048.07			
Total	177685.88	23				

The F value of 147.54 is higher than the critical F value of 4.3, indicating that the level of protease produced by the *prtAΔ* strains is significantly lower than the level of protease produced by the wildtype strains.

SUMMARY

The *prtAΔ* strains, MK189 and MK190, produce significantly less protease activity than the wildtype strains, MH2 and MK191, when incubated for 20 hours in sulphur-limiting conditions.

Appendix 2.

Raw data and statistical analysis of the levels of protease produced by the *prtAΔ* strains, MK189 and MK190, and the wildtype strains, MH2 and MK191, under nitrogen-limiting conditions.

Table A2.1. Raw data. (arbitrary units protease activity/gram dry weight mycelia)

Strain		MH2		MK191		MK189		MK190	
conditions		+N	-N	+N	-N	+N	-N	+N	-N
Assay	1.1	2.36	46.48	9.41	87.51	2.21	10.15	2.24	16.62
	1.2	3.26	79.54	5.08	117.69	0.98	12.83	7.16	12.92
	2.1	3.14	62.5	5.46	101.53	7.10	11.64	2.81	6.22
	2.2	2.82	59.42	2.54	80.60	4.56	9.86	2.14	12.31
	3.1	5.41	40.17	1.88	52.90	4.84	9.4	1.08	10.77
	3.2	3.77	53.08	7.6	68.65	5.33	6.87	2.61	6.57
	mean	**3.46**	**56.86**	**5.33**	**84.81**	**4.17**	**10.12**	**3.01**	**10.90**
	SD	±0.97	±12.61	±2.63	±21.08	±2.02	±1.86	±1.94	±3.64

N.B. Assays were performed in triplicate, with internal duplicates. Assay numbering refers to assay number: duplicate number. Conidia was inoculated into minimal medium containing 1% glucose as a carbon source, 10 mM ammonium tartrate as a nitrogen source and grown for 16 hours prior to transfer to either nitrogen-repressing medium (+N = minimal media containing 1% glucose, 10 mM ammonium tartrate), or nitrogen-derepressing medium (-N = minimal media containing 1% glucose, no nitrogen source), for 4 hours. All media used in this experiment was made from sulphate containing salt solution.

Under conditions which repress extracellular protease production (+N), all four strains produced very low levels of protease activity. Under these conditions the assay results are very similar between all four strains.

The *prtAΔ* strains produce much lower levels of protease than the wildtype strains, under nitrogen-nutrient-limiting (-N) conditions. The analysis of variance function (ANOVA) of Excel (Microsoft) was used to determine if the differences observed in the assays were significant.

ANOVA: single factor, comparing MH2 and MK191 (the wildtype strains).

SUMMARY

Groups	Count	Sum	Average	Variance
MH2	6	341.18	56.86	190.68
MK191	6	508.88	84.81	533.06

ANOVA

Source of variation	SS	df	MS	**F**	P-value	**F crit**
Between groups	2343.66	1	2343.66	**6.477**	0.029	**4.96**
Within groups	3618.66	10	361.87			
Total	5962.33	11				

The F value of 6.477 is higher than the critical F value of 4.96, indicating that there is a significant difference between the MH2 and MK191 strains.

ANOVA: single factor, comparing MK189 and MK190 (the *prtAΔ* strains).

SUMMARY

Groups	Count	Sum	Average	Variance
MK189	6	60.73	10.12	4.16
MK190	6	65.42	10.90	15.90

ANOVA

Source of variation	SS	df	MS	**F**	P-value	**F crit**
Between groups	1.83	1	1.83	**0.18**	0.68	**4.96**
Within groups	100.30	10	10.03			
Total	102.13	11				

The F value of 0.18 is lower than the critical F value of 4.96, indicating that there is no significance between the MK189 and MK190 strains.

As there was a significant difference between the two wildtype strains they cannot be combined, therefore all strains must be compared separately.

ANOVA: single factor, comparing MH2 and MK189 strains.

SUMMARY

Groups	Count	Sum	Average	Variance
MH2	6	341.18	56.86	190.68
MK189	6	60.73	10.12	4.16

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	6554.21	1	6554.21	**67.28**	9.46×10^{-6}	**4.96**
Within groups	974.20	10	97.42			
Total	7528.41	11				

The F value of 67.28 is higher than the critical F value of 4.96, indicating that the level of protease produced by the MK189 strain is significantly lower than the level of protease produced by the MH2 strain.

ANOVA: single factor, comparing MH2 and MK190 strains.

SUMMARY

Groups	Count	Sum	Average	Variance
MH2	6	341.18	56.86	190.68
MK190	6	65.42	10.90	15.90

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	6336.92	1	6336.92	**61.35**	1.42×10^{-5}	**4.96**
Within groups	1032.87	10	103.29			
Total	7369.79	11				

The F value of 61.35 is higher than the critical F value of 4.96, indicating that difference between the level of protease produced by the MK190 strain is significantly lower than the level of protease produced by the MH2 strain.

ANOVA: single factor, comparing MK191 and MK189 strains.

SUMMARY

Groups	Count	Sum	Average	Variance
MK191	6	508.88	84.81	533.06
MK189	6	60.73	10.12	4.16

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	16736.46	1	16736.46	**62.31**	1.32×10^{-5}	**4.96**
Within groups	2686.09	10	268.61			
Total	19422.55	11				

The F value of 62.31 is higher than the critical F value of 4.96, indicating that difference between the level of protease produced by the MK189 strain is significantly lower than the level of protease produced by the MK191 strain.

ANOVA: single factor, comparing MK191 and MK190 strains.

SUMMARY

Groups	Count	Sum	Average	Variance
MK191	6	508.88	84.81	533.06
MK190	6	65.42	10.90	15.90

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	16388.14	1	16388.14	**59.71**	1.59×10^{-5}	**4.96**
Within groups	2744.76	10	274.48			
Total	19132.89	11				

The F value of 59.71 is higher than the critical F value of 4.96, indicating that the level of protease produced by the MK190 strain is significantly lower than the level of protease produced by the MK191 strain.

SUMMARY

The statistical analysis has shown that though there is a significant difference between the two wildtype strains, the *prtAΔ* strains produce significantly less protease activity than both the wildtype strains.

Appendix 3.

Raw data and statistical analysis of the levels of protease produced by the *prtAΔ* strains, MK189 and MK190, and the wildtype strains, MH2 and MK191, under carbon-nutrient-limiting conditions.

Table A.3.1. Raw data. (arbitrary units protease activity/gram dry weight mycelia)

Strain		**MH2**		**MK191**		**MK189**		**MK190**	
conditions		**+C**	**-C**	**+C**	**-C**	**+C**	**-C**	**+C**	**-C**
Assay	1.1	0.94	112.80	3.42	123.92	10.25	91.00	1.76	45.69
	1.2	1.97	120.87	6.51	131.57	5.38	86.22	2.91	30.50
	2.1	1.41	93.29	3.30	149.06	5.50	84.07	5.26	65.42
	2.2	0.95	135.23	4.90	201.83	4.46	95.61	9.16	67.10
	3.1	1.53	168.94	3.70	210.87	2.80	71.12	3.31	70.72
	3.2	1.16	169.87	3.60	237.87	1.57	91.57	2.02	106.67
	mean	**1.33**	**133.50**	**4.23**	**175.85**	**4.99**	**86.60**	**4.07**	**64.35**
	SD	±0.40	±30.94	±1.26	±47.17	±3.00	±8.62	±2.79	±25.82

N.B. Assays were performed in triplicate, with internal duplicates. Assay numbering refers to assay number: duplicate number. Conidia was inoculated into minimal media containing 1% glucose as a carbon source, 10 mM ammonium tartrate as a nitrogen source, and grown for 16 hours prior to transfer to either carbon-repressing media, (+C = 10 mM ammonium tartrate, 1% glucose), or carbon-derepressing media, (-C = 10 mM ammonium tartrate, no carbon source added), for 16 hours. The minimal media used in this experiment was made using salt solution containing sulphate.

Under conditions which repress extracellular protease production (+C), all four strains produced very low levels of protease activity. Under these conditions the assay results were very similar between all four strains.

The *prtAΔ* strains produce much lower levels of protease than the wildtype strains, under carbon-nutrient limiting conditions (-C). The analysis of variance function (ANOVA) of Excel (Microsoft) was used to determine if the differences observed in the assays, which examined the protease levels in filtrate from cultures subjected to 16 hours carbon limitation, was significant.

ANOVA: single factor, comparing MH2 and MK191 (the wildtype strains).

SUMMARY

Groups	Count	Sum	Average	Variance
MH2	6	800.99	133.50	957.23
MK191	6	1055.11	175.85	2224.65

ANOVA

Source of variation	SS	df	MS	**F**	P-value	**F crit**
Between groups	5381.42	1	5381.42	**3.38**	0.096	**4.96**
Within groups	15909.37	10	1590.94			
Total	21290.79	11				

The F value of 3.38 is lower than the critical F value of 4.96, indicating that there is no significant difference between the MH2 and MK191 strains.

ANOVA: single factor, comparing MK189 and MK190 (the *prtAΔ* strains).

SUMMARY

Groups	Count	Sum	Average	Variance
MK189	6	519.59	86.60	74.26
MK190	6	386.10	64.35	666.81

ANOVA

Source of variation	SS	df	MS	**F**	P-value	**F crit**
Between groups	1484.90	1	1484.90	**4.01**	0.07	**4.96**
Within groups	3705.33	10	370.53			
Total	5190.23	11				

The F value of 4.01 is lower than the critical F value of 4.96, indicating that there is no significance between the MK189 and MK190 strains.

As there was a no significant difference in the data for the two wildtype strains they can be combined. No significant difference exists between the data for the two *prtAΔ* strains so they can be combined. The combined wildtype and combined *prtAΔ* groups can be compared directly.

ANOVA: single factor, comparing wildtype and *prtAΔ* strains.

SUMMARY

Groups	Count	Sum	Average	Variance
wildtype	12	1856.1	154.68	1935.53
prtA.Δ	12	905.69	75.47	471.84

ANOVA

Source of variation	SS	df	MS	**F**	P-value	**F crit**
Between groups	37637.03	1	37637.03	**31.27**	1.27×10^{-5}	**4.3**
Within groups	26281.01	22	1203.68			
Total	64118.04	23				

The F value of 31.27 is higher than the critical F value of 4.3, indicating that the level of protease produced by the *prtAΔ* strains is significantly lower than the level of protease produced by the wildtype strains.

SUMMARY.

The *prtAΔ* strains produce significantly less protease than the wildtype strains under carbon-derepressing conditions.

Appendix 4.

Raw data and statistical analysis of the levels of protease produced by the *prtAΔ* strains, MK189 and MK191, and the wildtype strains, MH2 and MK191, comparing the effects of exogenous protein and time.

Table A4.1. Raw data from protease assays examining filtrate from cultures after 4 hours in nitrogen-repressing or derepressing conditions, with or without 1% skim milk. (arbitrary units protease activity/gram dry weight mycelia).

strain	media	Assay 1.	Assay 2.	Assay 3.	Mean	SD
MH2	+N	10.60	12.31	0.65	**7.85**	±6.30
	+N +SM	6.11	8.16	0.50	**4.92**	±3.97
	-N	70.00	42.27	34.96	**49.07**	±18.48
	-N +SM	97.46	55.25	42.31	**65.01**	±28.84
MK191	+N	1.30	3.61	3.84	**2.92**	±1.41
	+N +SM	4.01	3.36	2.38	**3.25**	±0.82
	-N	64.15	66.72	39.07	**56.64**	±15.28
	-N +SM	79.12	77.71	58.21	**71.68**	±11.69
MK189	+N	7.13	5.70	4.82	**5.88**	±1.17
	+N +SM	5.86	0.15	2.32	**2.77**	±2.88
	-N	15.36	13.06	6.47	**11.63**	±4.62
	-N +SM	52.71	15.48	10.73	**26.31**	±22.99
MK190	+N	4.21	0.50	2.39	**2.37**	±1.85
	+N +SM	6.23	0.16	3.74	**3.38**	±3.05
	-N	12.90	18.11	3.41	**11.47**	±7.46
	-N +SM	22.74	26.73	11.42	**20.30**	±7.95

N.B. Assays were performed in triplicate. Conidia were inoculated into minimal media (containing 1% glucose, 10 mM ammonium tartrate) and grown for 16 hours prior to transfer to either nitrogen-repressing medium, (+N = 10 mM ammonium tartrate, 1% glucose), or nitrogen-derepressing medium, (-N = no nitrogen source added, 1% glucose), for 4 hours. +SM = 1% skim milk. Minimal media used in this experiment was made from a salt solution containing sulphate.

Table A4.1. Raw data from protease assays examining filtrate from cultures after 24 hours in nitrogen-repressing or derepressing conditions, with or without 1% skim milk. (arbitrary units protease activity/gram dry weight mycelia).

strain	media	Assay 1.	Assay 2.	Assay 3.	Mean	SD
MH2	+N	5.34	4.89	4.71	**4.98**	±0.33
	+N +SM	5.94	4.09	9.57	**6.53**	±2.79
	-N	84.16	29.22	55.45	**56.27**	±27.48
	-N +SM	103.20	51.71	60.57	**71.83**	±27.53
MK191	+N	10.61	11.81	5.22	**9.21**	±3.51
	+N +SM	7.49	7.85	3.33	**6.22**	±2.52
	-N	75.27	50.01	20.85	**48.71**	±27.23
	-N +SM	62.71	78.60	41.09	**60.80**	±18.83
MK189	+N	2.64	4.70	1.15	**2.83**	±1.78
	+N +SM	0.89	9.2	1.24	**3.77**	±4.70
	-N	44.23	16.1	4.59	**21.64**	±20.39
	-N +SM	62.08	29.38	14.03	**35.16**	±24.54
MK190	+N	8.25	16.94	9.35	**11.51**	±4.73
	+N +SM	5.33	8.03	10.55	**7.97**	±2.61
	-N	9.63	31.33	4.68	**15.21**	±14.17
	-N +SM	49.80	48.66	35.79	**44.75**	±7.78

N.B. Assays were performed in triplicate. Conidia were inoculated into minimal medium (containing 1% glucose, 10 mM ammonium tartrate) and grown for 16 hours prior to transfer to either nitrogen-repressing medium, (+N = 10 mM ammonium tartrate, 1% glucose), or nitrogen-derepressing medium, (-N = no nitrogen source added, 1% glucose), for 24 hours. +SM = 1% skim milk. Minimal medium used in this experiment was made from a sulphate-containing salt solution.

Under conditions which repress extracellular protease production (+N), all four strains produced very low levels of protease activity. Under these conditions the assay results were very similar between all four strains.

The *prtAΔ* strains produced much lower levels of protease than the wildtype strains, under nitrogen-limiting conditions (-N). Results of assays on solid media where skim milk is the sole nitrogen source have shown that the wildtype and *prtAΔ* strains have identical phenotypes, *i.e.* both produce a halo of protease activity of approximately equal size. Therefore the results of the solid and liquid assays for protease production are contradictory. The factors which differ between the two experiments are: 1) presence/absence of exogenous protein (skim milk), and 2) the length of time the strains were exposed to nutrient-limiting conditions (4 hours compared to 48 hours). This experiment was designed to test these two variables to determine the cause of the inconsistent results. The analysis of variance function (ANOVA) of Excel (Microsoft) was used to determine if the differences observed in the assays was significant.

- **Comparison of wildtype strains.**

ANOVA: single factor, the protease levels produced by MH2 compared to the protease levels produced by MK191, after 4 hours incubation in medium containing ammonium as the sole nitrogen source.

SUMMARY

Groups	Count	Sum	Average	Variance
MH2	3	23.55	7.85	39.66
MK191	3	8.76	2.92	1.98

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	36.50	1	36.50	**1.75**	0.26	**7.71**
Within groups	83.28	4	20.82			
Total	119.78	5				

The F value of 1.75 is lower than the critical F value of 7.71, indicating that there is no significant difference between the level of protease produced by MH2 and MK191 after 4 hours in medium containing ammonium. These results can be pooled.

ANOVA: single factor, the protease levels produced by MH2 compared to the protease levels produced by MK191, after 4 hours incubation in medium containing ammonium and skim milk as nitrogen sources.

SUMMARY

Groups	Count	Sum	Average	Variance
MH2	3	14.76	4.92	15.73
MK191	3	9.74	3.25	0.67

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	4.19	1	4.19	**0.51**	0.51	**7.71**
Within groups	32.80	4	8.20			
Total	36.99	5				

The F value of 0.51 is higher than the critical F value of 7.71, indicating that the levels of protease produced by MH2 and MK191 after 4 hours in medium containing ammonium and skim milk are not significantly different. These results can be pooled.

ANOVA: single factor, the protease levels produced by MH2 compared to the protease levels produced by MK191, after 4 hours incubation in medium containing no nitrogen source.

SUMMARY

Groups	Count	Sum	Average	Variance
MH2	3	147.22	49.07	341.44
MK191	3	169.93	56.64	233.37

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	85.93	1	85.93	**0.30**	0.61	**7.71**
Within groups	1149.61	4	287.40			
Total	1235.54	5				

The F value of 0.30 is lower than the critical F value of 7.71, indicating that the difference between the level of protease produced by MH2 and MK191, after 4 hours incubation in medium without nitrogen, is not significantly different. These results can be pooled.

ANOVA: single factor, the protease levels produced by MH2 compared to the protease levels produced by MK191, after 4 hours incubation in medium containing skim milk as the sole nitrogen source.

SUMMARY

Groups	Count	Sum	Average	Variance
MH2	3	195.02	65.01	831.62
MK191	3	215.04	71.68	136.62

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	66.79	1	66.79	**0.14**	0.73	**7.71**
Within groups	1936.47	4	484.12			
Total	2003.26	5				

The F value of 0.14 is lower than the critical F value of 7.71, indicating that the levels of protease produced by the MH2 and MK191 after 4 hours incubation in medium containing only skim milk as a nitrogen source, were not significantly different. These results can be pooled.

ANOVA: single factor, the protease levels produced by MH2 compared to the protease levels produced by MK191, after 24 hours incubation in medium containing ammonium as the sole nitrogen source.

SUMMARY

Groups	Count	Sum	Average	Variance
MH2	3	14.94	4.98	0.11
MK191	3	27.64	9.21	12.32

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	26.88	1	26.88	**4.33**	0.12	**7.71**
Within groups	24.85	4	6.21			
Total	51.74	5				

The F value of 4.33 is lower than the critical F value of 7.71, indicating that the difference between the levels of protease produced by MH2 and MK191 after 24 hours incubation in media containing ammonium, is not significantly different. These results can be pooled.

ANOVA: single factor, the protease levels produced by MH2 compared to the protease levels produced by MK191, after 24 hours incubation in medium containing ammonium and skim milk as nitrogen sources.

SUMMARY

Groups	Count	Sum	Average	Variance
MH2	3	19.60	6.53	7.78
MK191	3	18.67	6.22	6.32

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	0.14	1	0.14	**0.02**	0.89	**7.71**
Within groups	28.22	4	7.05			
Total	28.36	5				

The F value of 0.02 is lower than the critical F value of 7.71, indicating that the difference between the levels of protease produced by MH2 and MK191 after 24 hours incubation in medium containing ammonium and skim milk, is not significantly different. These results can be pooled.

ANOVA: single factor, the protease levels produced by MH2 compared to the protease levels produced by MK191, after 24 hours incubation in medium containing no nitrogen source.

SUMMARY

Groups	Count	Sum	Average	Variance
MH2	3	168.82	56.27	754.92
MK191	3	146.13	48.71	741.66

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	85.85	1	85.85	**0.11**	0.75	**7.71**
Within groups	2993.16	4	748.29			
Total	3079.01	5				

The F value of 0.11 is lower than the critical F value of 7.71, indicating that the levels of protease produced by MH2 and MK191 after 24 hours incubation in medium containing no nitrogen, were not significantly different. These results can be pooled.

ANOVA: single factor, the protease levels produced by MH2 compared to the protease levels produced by MK191, after 24 hours incubation in medium containing skim milk as the sole nitrogen source.

SUMMARY

Groups	Count	Sum	Average	Variance
MH2	3	215.48	71.83	758.11
MK191	3	182.40	60.80	354.39

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	182.31	1	189.31	**0.33**	0.60	**7.71**
Within groups	2225.00	4	556.25			
Total	2407.3	5				

The F value of 0.33 is lower than the critical F value of 7.71, indicating that difference between the levels of protease produced by MH2 and MK191 after 24 incubation in medium where skim milk is the sole nitrogen source, is not significantly different. These results can be pooled.

- **Comparison of *prtAΔ* strains.**

ANOVA: single factor, comparing the levels of protease produced by MK189 and MK190, after 4 hours incubation in medium containing ammonium as the sole nitrogen source.

SUMMARY

Groups	Count	Sum	Average	Variance
MK189	3	8.76	2.92	1.98
MK190	3	7.10	2.37	3.43

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	0.46	1	0.46	**0.17**	0.70	**7.71**
Within groups	10.82	4	2.71			
Total	11.28	5				

The F value of 0.17 is lower than the critical F value of 7.71, indicating that there is no significant difference between the levels of protease produced by MK189 and MK190 after 4 hours incubation in medium containing ammonium. These results can therefore be pooled.

ANOVA: single factor, comparing the levels of protease produced by MK189 and MK190, after 4 hours incubation in medium containing ammonium and skim milk as nitrogen sources.

SUMMARY

Groups	Count	Sum	Average	Variance
MK189	3	8.32	2.77	8.30
MK190	3	10.13	3.38	9.29

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	0.55	1	0.55	**0.82**	0.82	**7.71**
Within groups	35.18	4	8.80			
Total	35.73	5				

The F value of 0.82 is lower than the critical F value of 7.71, indicating that there is no significant difference between the levels of protease produced by MK189 and MK190 after 4 hours incubation in medium containing ammonium and skim milk. Therefore these results can be pooled.

ANOVA: single factor, comparing the levels of protease produced by MK189 and MK190, after 4 hours incubation in medium containing no nitrogen.

SUMMARY

Groups	Count	Sum	Average	Variance
MK189	3	34.89	11.63	21.30
MK190	3	34.41	11.47	55.57

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	0.04	1	0.04	**0.1×10^{-3}**	0.98	**7.71**
Within groups	153.75	4	38.44			
Total	153.78	5				

The F value of 0.1×10^{-3} is lower than the critical F value of 7.71, indicating that the levels of protease produced by MK189 and MK190 after 4 hours incubation in medium containing no nitrogen source, are not significantly different. These results can be pooled.

ANOVA: single factor, comparing the levels of protease produced by MK189 and MK190, after 4 hours incubation in medium containing skim milk as the sole nitrogen source.

SUMMARY

Groups	Count	Sum	Average	Variance
MK189	3	78.92	26.31	528.43
MK190	3	60.89	20.30	63.15

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	54.17	1	54.17	**0.18**	0.69	**7.71**
Within groups	1183.14	4	295.79			
Total	1237.31	5				

The F value of 0.18 is lower than the critical F value of 7.71, indicating that the levels of protease produced by MK189 and MK190 after 4 hours incubation in medium containing skim milk as the sole nitrogen source, are not significantly different. These results can be pooled.

ANOVA: single factor, comparing the levels of protease produced by MK189 and MK190, after 24 hours incubation in medium containing ammonium as the sole nitrogen source.

SUMMARY

Groups	Count	Sum	Average	Variance
MK189	3	8.49	2.83	3.16
MK190	3	34.54	11.51	22.39

ANOVA

Source of variation	SS	df	MS	**F**	P-value	**F crit**
Between groups	113.07	1	113.07	**8.85**	0.04	**7.71**
Within groups	51.11	4	12.78			
Total	164.17	5				

The F value of 8.85 is higher than the critical F value of 7.71, indicating that the levels of protease produced by MK189 and MK190 after 4 hours incubation in medium containing ammonium as the sole nitrogen source, are significantly different. These results cannot be pooled.

ANOVA: single factor, comparing the levels of protease produced by MK189 and MK190, after 24 hours incubation in medium containing ammonium and skim milk as nitrogen sources.

SUMMARY

Groups	Count	Sum	Average	Variance
MK189	3	11.33	3.78	22.09
MK190	3	23.91	7.97	6.81

ANOVA

Source of variation	SS	df	MS	**F**	P-value	**F crit**
Between groups	26.38	1	26.37	**1.83**	0.25	**7.71**
Within groups	57.81	4	14.45			
Total	84.19	5				

The F value of 1.83 is lower than the critical F value of 7.71, indicating that difference between the levels of protease produced by MK189 and MK190, after 24 hours incubation in medium containing ammonium and skim milk as nitrogen sources, are not significantly different. These results can be pooled.

ANOVA: single factor, comparing the levels of protease produced by MK189 and MK190, after 24 hours incubation in medium containing no nitrogen source.

SUMMARY

Groups	Count	Sum	Average	Variance
MK189	3	64.92	21.64	415.85
MK190	3	45.64	15.21	200.94

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	61.95	1	61.95	**0.20**	0.68	**7.71**
Within groups	1233.57	4	308.39			
Total	1295.53	5				

The F value of 0.20 is lower than the critical F value of 7.71, indicating that there is no significant difference between the level of protease produced by MK189 and MK190 after 24 hours incubation in medium containing no nitrogen source.

ANOVA: single factor, comparing the levels of protease produced by MK189 and MK190, after 24 hours incubation in medium containing no nitrogen source.

SUMMARY

Groups	Count	Sum	Average	Variance
MK189	3	105.49	35.16	602.29
MK190	3	134.25	44.75	60.54

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	137.86	1	137.86	**0.42**	0.55	**7.71**
Within groups	1325.64	4	331.41			
Total	1463.50	5				

The F value of 0.42 is lower than the critical F value of 7.71, indicating that there is a no significant difference between the level of protease produced by MK189 and MK190, after 24 hours incubation in medium containing skim milk as the sole nitrogen source.

- **Comparison of the wildtype strains, MH2 and MK191, and the *prtAΔ* strains, MK189 and MK190.**

ANOVA: single factor, comparing wildtype and *prtAΔ* strains after 4 hours incubation in medium containing no nitrogen source.

SUMMARY

Groups	Count	Sum	Average	Variance
wildtype	6	317.15	52.86	247.11
prtAΔ	6	69.30	11.55	30.76

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	5118.97	1	5118.97	**36.85**	1.2×10^{-4}	**4.96**
Within groups	1389.32	10	138.93			
Total	6508.29	11				

The F value of 36.85 is higher than the critical F value of 4.96, indicating that the level of protease produced by the wildtype strains after 4 hours incubation in nitrogen-limiting conditions, is significantly different from the level produced by the *prtAΔ* strains after 4 hours incubation in nitrogen-limiting conditions.

ANOVA: single factor, comparing wildtype and *prtAΔ* strains after 4 hours incubation in medium containing skim milk as the sole nitrogen source.

SUMMARY

Groups	Count	Sum	Average	Variance
MK189	6	410.05	68.34	400.65
MK190	6	139.81	23.30	247.46

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	6085.94	1	6085.94	**18.78**	1.5×10^{-3}	**4.96**
Within groups	3240.57	10	324.06			
Total	9326.51	11				

The F value of 18.78 is higher than the critical F value of 4.96, indicating that difference between the level of protease produced by the wildtype strains is significantly different from the levels of protease produced by the *prtAΔ* strains after 4 hours incubation in medium containing skim milk as the sole nitrogen source.

ANOVA: single factor, comparing wildtype and *prtAΔ* strains after 24 hours incubation in medium containing no nitrogen source.

SUMMARY

Groups	Count	Sum	Average	Variance
wildtype	6	314.95	52.49	615.80
prtAΔ	6	110.56	18.43	259.11

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	3481.27	1	3481.27	**7.96**	0.02	**4.96**
Within groups	4374.54	10	437.45			
Total	7855.81	11				

The F value of 7.96 is higher than the critical F value of 4.96, indicating that the level of protease produced by the wildtype strains after 24 hours incubation in medium containing no nitrogen source, is significantly different from the levels of protease levels produced by the *prtAΔ* strains after 24 hours incubation in medium containing no nitrogen source.

ANOVA: single factor, comparing wildtype and *prtAΔ* strains after 24 hours incubation in medium containing skim milk as the sole nitrogen source.

SUMMARY

Groups	Count	Sum	Average	Variance
wildtype	6	397.88	66.31	481.46
prtAΔ	6	239.74	39.96	292.70

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	2083.92	1	2083.92	**5.38**	0.04	**4.96**
Within groups	3870.82	10	387.08			
Total	5954.74	11				

The F value of 5.38 is higher than the critical F value of 4.96, indicating that the level of protease produced by the wildtype strains after 24 hours incubation in medium containing skim milk as the sole nitrogen source, is significantly different to the levels of protease produced by the *prtAΔ* strains after 24 hours incubation in medium containing skim milk as the sole nitrogen source.

- **Comparison of the effects of the length of time that the wildtype strains, MH2 and MK191, and the *prtAΔ* strains, MK189 and MK190, were incubated under the experimental conditions.**

ANOVA: single factor, comparing the levels of protease produced by the wildtype strains, MH2 and MK191, after 4 hours or 24 hours incubation in medium containing ammonium as a nitrogen source.

SUMMARY

Groups	Count	Sum	Average	Variance
4 hours	6	32.31	5.39	23.96
24 hours	6	42.58	7.10	10.35

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	8.79	1	8.79	**0.51**	0.49	**4.96**
Within groups	171.51	10	17.15			
Total	180.30	11				

The F value of 0.51 is lower than the critical F value of 4.96, indicating that there is no significant difference between the level of protease produced by the wildtype strains, MH2 and MK191, after 4 or 24 hours incubation in media containing ammonium as a nitrogen source.

ANOVA: single factor, comparing the levels of protease produced by the wildtype strains, MH2 and MK191, after 4 hours or 24 hours incubation in medium containing ammonium and skim milk as nitrogen sources.

SUMMARY

Groups	Count	Sum	Average	Variance
4 hours	6	24.51	4.08	7.40
24 hours	6	38.27	6.38	5.67

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	15.78	1	15.78	**2.42**	0.15	**4.96**
Within groups	65.35	10	6.54			
Total	81.14	11				

The F value of 2.42 is lower than the critical F value of 4.96, indicating that there is no significant difference between the level of protease produced by the wildtype strains, MH2 and MK191, after 4 or 24 hours incubation in medium containing ammonium and skim milk as nitrogen sources.

ANOVA: single factor, comparing the levels of protease produced by the wildtype strains, MH2 and MK191, after 4 hours or 24 hours incubation in medium containing no nitrogen source.

SUMMARY

Groups	Count	Sum	Average	Variance
4 hours	6	317.15	52.86	247.11
24 hours	6	314.95	52.49	615.80

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	0.40	1	0.40	**9.3×10^{-4}**	0.98	**4.96**
Within groups	4314.55	10	431.45			
Total	4314.95	11				

The F value of 9.3×10^{-4} is lower than the critical F value of 4.96, indicating that there is no significant difference between the level of protease produced by the wildtype strains, MH2 and MK191, after 4 or 24 hours incubation in medium containing no nitrogen source.

ANOVA: single factor, comparing the levels of protease produced by the wildtype strains, MH2 and MK191, after 4 hours or 24 hours incubation in medium containing skim milk as the sole nitrogen source.

SUMMARY

Groups	Count	Sum	Average	Variance
4 hours	6	410.05	68.34	400.65
24 hours	6	397.88	66.31	481.46

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	12.35	1	12.35	**0.03**	0.87	**0.496**
Within groups	4410.58	10	441.06			
Total	4422.93	11				

The F value of 0.03 is lower than the critical F value of 4.96, indicating that there is no significant difference between the level of protease produced by the wildtype strains, MH2 and MK191, after 4 or 24 hours incubation in medium containing skim milk as the sole nitrogen source.

ANOVA: single factor, comparing the levels of protease produced by the *prtAΔ* strains, MK189 and MK190, after 4 hours or 24 hours incubation in media containing ammonium as the sole nitrogen source.

As the two mutant strains were found to produce significantly different levels of protease activity, when incubated for 24 hours in media containing ammonium as the sole nitrogen source, each strain must be compared separately.

ANOVA: single factor, comparing MK189 after 4 or 24 hours incubation in medium containing ammonium as the sole nitrogen source.

SUMMARY

Groups	Count	Sum	Average	Variance
4 hours	3	17.65	5.88	1.37
24 hours	3	8.49	2.83	3.16

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	13.99	1	13.99	**6.18**	0.07	**7.71**
Within groups	9.06	4	2.27			
Total	23.06	5				

The F value of 6.18 is lower than the critical F value of 7.71, indicating that there is no significant difference between the level of protease produced by MK189 after 4 or 24 hours incubation in medium containing ammonium as the sole nitrogen sources.

ANOVA: single factor, comparing MK190 after 4 or 24 hours incubation in medium containing ammonium as the sole nitrogen source.

SUMMARY

Groups	Count	Sum	Average	Variance
4 hours	3	7.10	2.37	3.43
24 hours	3	34.54	11.51	22.39

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	125.47	1	125.47	**9.72**	0.04	**7.71**
Within groups	51.65	4	12.91			
Total	177.12	5				

The F value of 9.72 is higher than the critical F value of 7.71, indicating that there is a significant difference between the level of protease produced by MK190 after 4 hours compared to 24 hours incubation, in medium containing ammonium as the sole nitrogen sources.

ANOVA: single factor, comparing the levels of protease produced by the *prtAΔ* strains, MK189 and MK190, after 4 hours or 24 hours incubation in medium containing ammonium and skim milk as nitrogen sources.

SUMMARY

Groups	Count	Sum	Average	Variance
4 hours	6	18.45	3.08	7.15
24 hours	6	35.24	5.87	16.84

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	23.49	1	23.49	**1.96**	0.19	**4.96**
Within groups	119.92	10	11.99			
Total	143.41	11				

The F value of 1.96 is lower than the critical F value of 4.96, indicating that there is no significant difference between the level of protease produced by the *prtAΔ* strains, MK189 and MK190, after 4 or 24 hours incubation in medium containing ammonium and skim milk as nitrogen sources.

ANOVA: single factor, comparing the levels of protease produced by the *prtAΔ* strains, MK189 and MK190, after 4 hours or 24 hours incubation in medium containing no nitrogen source.

SUMMARY

Groups	Count	Sum	Average	Variance
4 hours	6	69.30	11.55	30.76
24 hours	6	110.56	18.43	259.11

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	141.87	1	141.87	**0.98**	0.34	**4.96**
Within groups	1449.31	10	144.93			
Total	1591.18	11				

The F value of 0.98 is lower than the critical F value of 4.96, indicating that there is no significant difference between the level of protease produced by the *prtAΔ* strains, MK189 and MK190, after 4 or 24 hours incubation in medium containing no nitrogen source.

ANOVA: single factor, comparing the levels of protease produced by the *prtAΔ* strains, MK189 and MK190, after 4 hours or 24 hours incubation in medium containing skim milk as the sole nitrogen source.

SUMMARY

Groups	Count	Sum	Average	Variance
4 hours	6	139.81	23.30	247.46
24 hours	6	239.74	39.96	292.70

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	832.20	1	832.20	**3.08**	0.11	**4.96**
Within groups	2700.81	10	270.08			
Total	3533.01	11				

The F value of 3.08 is lower than the critical F value of 4.96, indicating that there is no significant difference between the level of protease produced by the *prtAΔ* strains, MK189 and MK190, after 4 or 24 hours incubation in medium containing skim milk as the sole nitrogen source.

- **Comparison of the effects of exogenous protein on the levels of protease produced by the wildtype strains, MH2 and MK191, and the *prtAΔ* strains, MK189 and MK190.**

ANOVA: single factor, comparing the levels of protease produced by the wildtype strains, MH2 and MK191, after 4 hours incubation in medium containing ammonium as a nitrogen source, with or without skim milk.

SUMMARY

Groups	Count	Sum	Average	Variance
no skim milk	6	32.31	5.39	23.96
skim milk	6	24.51	4.08	7.40

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	5.07	1	5.07	**0.32**	0.58	**4.96**
Within groups	156.77	10	15.68			
Total	161.84	11				

The F value of 0.32 is lower than the critical F value of 4.96, indicating that there is no significant difference between the level of protease produced by the wildtype strains, MH2 and MK191, after 4 incubation in medium containing ammonium as a nitrogen source, with or without skim milk.

ANOVA: single factor, comparing the levels of protease produced by the wildtype strains, MH2 and MK191, after 4 hours incubation in medium containing no ammonium, with or without skim milk.

SUMMARY

Groups	Count	Sum	Average	Variance
no skim milk	6	317.15	52.86	247.11
skim milk	6	410.05	68.34	400.65

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	719.28	1	719.28	**2.22**	0.17	**4.96**
Within groups	3238.80	10	323.88			
Total	3958.08	11				

The F value of 2.22 is lower than the critical F value of 4.96, indicating that there is no significant difference between the level of protease produced by the wildtype strains, MH2 and MK191, after 4 incubation in medium containing no ammonium, with or without skim milk.

ANOVA: single factor, comparing the levels of protease produced by the wildtype strains, MH2 and MK191, after 24 hours incubation in medium containing ammonium, with or without skim milk.

SUMMARY

Groups	Count	Sum	Average	Variance
no skim milk	6	42.58	7.10	10.35
skim milk	6	38.27	6.38	5.67

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	1.55	1	1.55	**0.19**	0.67	**4.96**
Within groups	80.10	10	80.01			
Total	81.65	11				

The F value of 0.19 is lower than the critical F value of 4.96, indicating that there is no significant difference between the level of protease produced by the wildtype strains, MH2 and MK191, after 24 hours incubation in medium containing ammonium, with or without skim milk.

ANOVA: single factor, comparing the levels of protease produced by the wildtype strains, MH2 and MK191, after 24 hours incubation in medium containing no ammonium, with or without skim milk as the sole nitrogen source.

SUMMARY

Groups	Count	Sum	Average	Variance
no skim milk	6	315.049	52.51	615.71
skim milk	6	397.88	66.31	481.46

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	571.69	1	571.69	**1.04**	0.33	**0.496**
Within groups	5485.85	10	548.58			
Total	6057.54	11				

The F value of 1.04 is lower than the critical F value of 4.96, indicating that there is no significant difference between the level of protease produced by the wildtype strains, MH2 and MK191, after 24 hours incubation in medium containing no nitrogen, with or without skim milk as the sole nitrogen source.

ANOVA: single factor, comparing the levels of protease produced by the *prtAΔ* strains, MK189 and MK190, after 4 hours incubation in medium containing ammonium as the sole nitrogen source, with or without skim milk.

SUMMARY

Groups	Count	Sum	Average	Variance
no skim milk	6	15.85	2.64	2.26
skim milk	6	18.45	3.08	7.15

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	0.56	1	0.56	**0.12**	0.74	**4.96**
Within groups	47.01	10	4.70			
Total	47.57	11				

The F value of 0.12 is lower than the critical F value of 4.96, indicating that there is no significant difference between the level of protease produced by the *prtAΔ* strains, MK189 and MK190, after 4 hours incubation in medium containing ammonium, with or without skim milk.

ANOVA: single factor, comparing the levels of protease produced by the *prtAΔ* strains, MK189 and MK190, after 4 hours incubation in medium containing no ammonium, with or without skim milk as the sole nitrogen source.

SUMMARY

Groups	Count	Sum	Average	Variance
no skim milk	6	69.30	11.55	30.76
skim milk	6	139.81	23.30	247.46

ANOVA

Source of variation	SS	df	MS	**F**	P-value	**F crit**
Between groups	414.28	1	414.28	**2.98**	0.12	**4.96**
Within groups	1391.09	10	139.11			
Total	1805.38	11				

The F value of 2.98 is lower than the critical F value of 4.96, indicating that there is no significant difference between the level of protease produced by the *prtAΔ* strains, MK189 and MK190, after 4 hours incubation in medium containing no nitrogen source, with or without skim milk.

ANOVA: single factor, comparing the levels of protease produced by the *prtAΔ* strains, MK189 and MK190, after 24 hours incubation in medium containing ammonium, with or without skim milk.

As the two mutant strains were found to produce significantly different levels of protease activity, when incubated for 24 hours in medium containing ammonium as the sole nitrogen source, each strain must be compared separately.

ANOVA: single factor, comparing the levels of protease produced by MK189 after 24 hours incubation in medium containing ammonium, with or without skim milk.

SUMMARY

Groups	Count	Sum	Average	Variance
no skim milk	3	8.49	2.83	3.16
skim milk	3	11.32	3.77	22.11

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	1.34	1	1.34	**0.11**	0.76	**7.71**
Within groups	50.54	4	12.64			
Total	51.88	5				

The F value of 0.11 is higher than the critical F value of 7.71, indicating that there is no significant difference between the level of protease produced by MK189 after 24 hours incubation in medium containing ammonium, with or without skim milk.

ANOVA: single factor, comparing the levels of protease produced by MK190 after 24 hours incubation in medium containing ammonium, with or without skim milk.

SUMMARY

Groups	Count	Sum	Average	Variance
no skim milk	3	34.54	11.51	22.39
skim milk	3	23.91	7.97	7.80

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	18.83	1	18.83	**1.29**	0.32	**7.71**
Within groups	58.37	4	14.59			
Total	77.20	5				

The F value of 1.29 is higher than the critical F value of 7.71, indicating that there is no significant difference between the level of protease produced by MK190 after 24 hours incubation in medium containing ammonium, with or without skim milk.

ANOVA: single factor, comparing the levels of protease produced by the *prtAΔ* strains, MK189 and MK190, after 24 hours incubation in medium containing no ammonium, with or without skim milk.

SUMMARY

Groups	Count	Sum	Average	Variance
no skim milk	6	110.56	18.43	259.11
skim milk	6	239.74	39.96	292.70

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	1390.62	1	1390.62	**5.04**	0.05	**4.96**
Within groups	2759.03	10	275.90			
Total	4149.65	11				

The F value of 5.04 is higher than the critical F value of 4.96, indicating that there is a significant difference between the level of protease produced by the *prtAΔ* strains, MK189 and MK190, after 24 hours incubation in medium containing no ammonium, with or without skim milk as the sole nitrogen source.

SUMMARY OF RESULTS.

Under nitrogen-limiting conditions there was a significant difference between the wildtype strains, MH2 and MK191, and the *prtAΔ* strains, MK189 and MK190, after 4 or 24 hours, with or without skim milk in the media.

Increasing the time of incubation did not result in a significant difference in the level of protease produced by the wildtype strains, MH2 and MK191, under any of the conditions tested. Statistical analysis has shown that there is a significant difference in the level of protease produced by MK190 in media containing ammonium as the sole nitrogen source. No significant difference was observed for the *prtAΔ* strains, MK189 and MK190, under any of the other conditions examined.

The presence of exogenous protein did not have a statistically significant effect on the level of protease activity produced by the wildtype strains, MH2 and MK191. The addition of skim milk to the *prtAΔ* strains, MK189 and MK190, grown for 24 hours in nitrogen-limiting conditions, did result in a statistically significant difference in the level of protease produced by these strains. The level of protease produced by the *prtAΔ* strains, MK189 and MK190, was not affected by the presence/absence of skim milk, under any of the other conditions tested.

Appendix 5.

Analysis of the haploids derived from MK189/J3.

The genotypes of the strains used to construct this diploid were:

MK189 = yA_1 $pabaA_1$; $argB_2$; $argB^+$:$prtA\Delta$

J3 = $AcrA_1$; $galA_1$; $pyroA_4$; hxA_1; sB_1; $nicB_8$; $riboB_2$

Table A5.1. Raw data (from haploids which were streaked for single colonies).

Chromosome number and marker.									
I yA_1	**II** $AcrA_1$	**III** $galA_1$	**IV** $pyroA_4$	**V** hxA_1	**VI** sB_1	**VII** $nicB_8$	**VIII** $riboB_2$	$prtA\Delta$	**№.**
+	-	+	-	+	-	-	+	-	2
+	+	-	-	-	-	+	+	+	2
+	+	+	-	+	-	-	+	-	1
+	-	-	-	-	-	+	-	+	1
+	-	-	-	-	-	+	+	+	1
+	-	-	+	-	-	+	+	+	1
+	+	-	-	+	-	+	+	-	2
+	-	-	-	+	-	+	+	-	1
-	+	+	-	+	-	-	-	-	1
-	+	-	-	-	-	+	+	+	4
-	-	+	+	+	-	-	-	-	1
-	-	-	+	+	+	-	-	-	1
-	+	-	-	-	+	+	-	+	1
-	+	-	-	+	-	-	+	-	1
-	+	+	+	+	+	+	-	-	1
-	-	-	+	+	-	+	+	-	1
-	-	-	+	-	-	-	+	+	1
								total	23

+ = wildtype allele. - = mutant allele.

Though these results are from a small data set (23 haploids), it can be seen from the data that the parental *prtA* allele and chromosome V markers are co-segregating, ie. haploids are either hxA^+ $prtA\Delta$ or hxA_1 $prtA^+$. *prtA* is not co-segregating with any other chromosome marker, therefore these results show unambiguously that *prtA* is linked to chromosome V.

Appendix 6.

Mapping of *prtA* to a region of chromosome V using a marker strain. Analysis of the cross between MK189 x A613.

Phenotypes of the strains used in this cross were:

MK189 = yA_1 $pabaA_1$; $argB_2$; $argB^+$;$prtA\Delta$

A613 = $AcrA_1$; $nicA_2$ pA_2 $facA_{303}$ hxA_1 $riboD_5$

Table A6.1. Raw data from MK189 x A613. As *prtA* had been mapped to chromosome V, only the chromosome V markers are included. The cross was scored for the other markers, which segregated as expected, and so are not included in this analysis.

Genetic marker						No
$nicA_2$	pA_2	$facA_{303}$	hxA_1	$riboD_5$	$prtA\Delta$	
+	+	+	+	+	-	41
-	-	-	-	-	+	17
-	+	+	+	+	-	16
+	+	+	+	-	-	16
-	-	-	-	+	-	14
+	+	-	-	-	+	11
+	+	-	-	+	-	10
+	-	+	+	+	-	10
+	-	-	-	-	+	9
-	-	+	+	+	-	9
-	+	-	-	-	+	9
+	-	-	-	+	-	8
-	+	+	+	-	-	8
-	-	-	+	+	-	6
+	+	+	+	-	+	6
+	+	-	-	+	+	4
+	-	+	-	-	+	4
+	+	+	+	+	+	3
-	-	-	-	-	-	3
-	-	-	-	+	+	3
-	-	+	+	-	-	3
+	+	+	-	-	+	3
+	+	+	-	+	-	3
-	-	+	+	-	+	3
-	+	-	-	+	-	3
+	-	-	-	-	-	2
+	-	+	-	+	+	2
-	+	+	+	+	+	2
+	-	-	+	+	-	2
-	-	+	-	-	+	2
-	+	+	+	-	+	2
+	-	-	-	+	+	2

Table A6.1 continued

Genetic marker						No
nicA	*pA*	*facA*	*hxA*	*riboD*	*prtA*	
-	+	-	-	+	+	2
+	-	+	+	-	-	2
+	+	-	+	+	-	2
-	+	-	+	+	-	2
-	+	-	-	-	-	1
-	+	+	-	-	+	1
+	-	+	+	+	+	1
-	-	+	-	+	-	1
+	-	+	+	-	+	1
-	+	-	+	-	+	1
+	+	+	-	+	+	1
-	-	-	+	-	+	1
+	-	-	+	-	-	1
-	-	+	+	+	+	1
-	-	+	-	+	+	1
+	-	+	-	+	-	1
					total	**258**

It is of note that there are less $prtA^+$ segregants than $prtA\Delta$ segregants. This is due to the presence of $argB^-$ in this cross. All $prtA\Delta$ segregants are viable as they contain the $argB^+$ selectable marker, whereas only half the $prtA^+$ segregants would be expected to be $argB^+$.

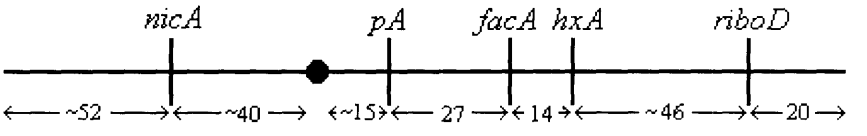

Figure A6.1. Diagram showing the position of the markers carried by strain A613 on chromosome V. Numbers shown refer to the published map distances in centi-Morgans (Clutterbuck, 1993).

To determine which of the chromosome V markers were linked to *prtA* the recombination frequency between *prtA* and the chromosome V marker genes were determined. Bracketed figures take into account the double recombinants.

- ***prtA* and *nicA***

parental genotypes:	*prtAΔ nicA*$^+$	100 (43)
	prtA$^+$ *nicA*$_2$	45 (17)
single recombinant genotypes:	*prtAΔnicA*$_2$	66
	prtA$^+$ *nicA*$^+$	47
	double recombinants:	85

$$RF = \frac{66 + 47}{258} = 0.44\ (1.10)$$

- ***prtA* and *pA***

parental genotypes:	*prtAΔ pA*$^+$	102 (57)
	prtA$^+$ *pA*$_1$	47 (27)
recombinant genotypes:	*prtAΔ pA*$_1$	62
	prtA$^+$ *pA*$^+$	47
	double recombinants	(65)

$$RF = \frac{62 + 47}{258} = 0.42\ (0.92)$$

- ***prtA* and *facA***

parental genotypes:	*prtAΔ facA*$^+$	110 (80)
	prtA$^+$ *facA*$_{303}$	59 (46)
recombinant genotypes:	*prtAΔ facA*$_{303}$	54
	prtA$^+$ *facA*$^+$	35
	double recombinants	(43)

$$RF = \frac{54 + 34}{258} = 0.34\ (0.68)$$

- ***prtA* and *hxA***

parental genotypes:	*prtAΔ hxA*$^+$	119
	prtA$^+$ *hxA*$_1$	71
recombinant genotypes:	*prtAΔ hxA*$_1$	46
	prtA$^+$ *hxA*$^+$	22

$$RF = \frac{46 + 22}{258} = 0.26$$

- ***prtA* and *riboD***

parental genotypes:	*prtAΔ riboD*$^+$	128
	prtA$^+$ *riboD*$_1$	70
recombinant genotypes:	*prtAΔ riboD*$_1$	36
	prtA$^+$ *riboD*$^+$	24

$$RF = \frac{36 + 24}{258} = 0.23$$

The results indicate that *prtA* is linked to *hxA* and *riboD*. After it had been established that *prtA* must be located between *hxA* and *riboD* (see below for explanation), the map distance between *hxA* and *riboD* was calculated taking into account double recombinants.

- ***hxA* and *riboD***

parental genotypes:	*hxA*$^+$ *riboD*$^+$	96(85)
	hxA$^-$ *riboD*$^-$	62(56)
recombinant genotypes:	*hxA*$^+$ *riboD*$^-$	54
	hxA$^-$ *riboD*$^+$	46
	double recombinants	(17)

$$RF = \frac{54 + 46}{258} = 0.39\ (0.52)$$

The published genetic map of chromosome V places *hxA* and *riboD* greater than 50 m.u. apart. My data places *hxA* and *riboD* 52 m.u. apart, therefore *prtA* must be located between these two genes.

- ***facA* and *hxA***

parental genotypes:	$facA^{+}hxA^{+}$	124
	$facA^{-}\ hxA^{-}$	97
recombinant genotypes:	$facA^{+}hxA^{-}$	19
	$facA^{-}\ hxA^{+}$	18

$$RF = \frac{19 + 18}{258} = 0.14$$

Appendix 7.

Mapping *prtA* to *lysE*.

The genotypes of the strains used in this cross were:

A296 = biA_1; $lysE_1$; sB_3

PVK10 = yA_1 $pabaA_1$ $AcrA_1$; $nicA_2$ pA_2 $facA_{303}$ hxA_1 $prtA\Delta$ $riboD_5$

Table A7.1. Raw Data. As *prtA* had been mapped to chromosome V, only the chromosome V markers are included. The cross was scored for the other markers, which segregated as expected and are not included in this analysis.

$nicA_2$	pA_2	$facA_{303}$	hxA_1	$lysE_1$	$riboD_5$	$prtA\Delta$	No. segregants
-	-	-	-	+	-	-	42
-	-	-	-	+	+	-	12
+	+	+	+	-	+	n/s	10
+	+	+	+	-	+	n/s	9
+	+	+	+	-	-	n/s	8
-	+	-	-	+	-	-	7
+	-	-	-	+	-	-	7
+	+	-	-	+	-	-	7
+	+	+	-	+	-	-	5
+	+	-	-	+	+	-	4
+	+	-	+	-	+	n/s	4
+	+	-	+	+	-	-	4
+	+	+	-	+	+	-	3
+	+	-	+	+	+	-	3
+	+	+	+	+	+	-	3
+	-	+	-	+	-	-	3
-	+	+	+	-	+	n/s	3
-	-	-	-	+	-	+	3
-	-	+	+	-	+	n/s	3
-	-	-	-	+	+	+	2
-	-	+	+	-	-	n/s	2
-	-	+	-	+	-	-	2
+	+	-	+	-	-	n/s	2
+	-	-	-	+	+	-	2
+	+	-	-	+	+	+	2
-	+	-	-	+	+	+	2
-	+	+	+	-	-	n/s	2
+	+	+	+	+	+	+	2
+	+	-	+	+	+	+	2
+	+	+	-	-	-	n/s	2
+	-	+	+	-	+	n/s	1
+	-	+	+	+	+	-	1
+	-	-	-	+	+	+	1
+	-	+	+	-	-	n/s	1
+	+	-	-	-	+	n/s	1
+	+	-	+	+	+	-	1
-	+	+	+	+	+	+	1

Table A7.1 continued

nicA	*pA*	*facA*	*hxA*	*lysE*	*riboD*	*prtA*	No. segregants
-	+	+	-	+	-	-	1
-	-	+	-	+	+	-	1
+	-	+	+	+	-	-	1
+	+	+	-	-	+	n/s	1
+	-	-	+	+	-	-	1
+	+	+	-	-	+	n/s	1
+	-	-	+	-	-	n/s	1
+	+	-	-	-	-	n/s	1
+	+	+	+	+	-	-	1
-	-	-	+	+	-	-	1
-	-	+	+	+	+	-	1
-	+	-	+	+	+	+	1
-	-	+	+	-	-	n/s	1
n/s = not scored							182

As lysine suppresses protease production, $lysE_l$ segregants could not be scored for *prtA*. Therefore, for calculations where the *prtA* phenotype needed to be known, data regarding only the 129 $lysE^+$ segregants was used.

The low numbers (less than 50%) of $prtA^+$ segregants observed in this cross is probably due to *argB*-segregating in this cross. There was a 50% chance that PVK10 was carrying the $argB^-$ allele (in addition to the ectopic $argB^+$ allele used to select the *prtAΔ* mutants).

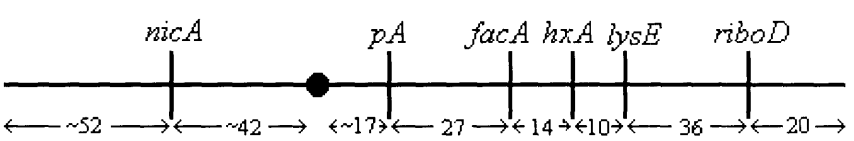

Figure A7.1. Diagram showing the position of the chromosome V markers carried by strain A296 and strain PVK10. Numbers shown refer to the published map distances in centi-Morgans (Clutterbuck, 1993).

Recombination frequencies were calculated for the genes in the *hxA* - *riboD* region. Bracketed figures take into account the double recombinants.

- ***prtA* and *hxA***

parentals:	$prtA^+$ $lysE^-$ hxA^+	not scorable
	$prtA^-$ $lysE^+$ hxA^-	96
single recombinants:	$prtA^+$ $lysE^+$ hxA^-	17
	$prtA^-$ $lysE^-$ hxA^+	not scorable
	$prtA^-$ $lysE^+$ hxA^+	10
	$prtA^+$ $lysE^-$ hxA^-	not scorable
double recombinants:	$prtA^+$ $lysE^+$ hxA^+	6
	$prtA^-$ $lysE^-$ hxA^-	not scorable

$$RF = \frac{17 + 10}{129} = 0.21\ (0.30)$$

- ***lysE* and *prtA***

parentals:	$lysE^+prtA^-$	116
	$lysE^-$ $prtA^+$	not scorable
recombinants:	$lysE^+$ $prtA^+$	13
	$lysE^-$ $prtA^-$	not scorable

$$RF = \frac{13}{129} = 0.10$$

- ***riboD* and *prtA***

parentals:	$riboD^+$ $prtA^+$	13
	$riboD^-$ $prtA^-$	82
recombinants:	$riboD^+$ $prtA^-$	31
	$riboD^-$ $prtA^+$	3

$$RF = \frac{31 + 3}{129} = 0.26$$

- ***hxA* and *lysE***

parentals:	hxA^+ $lysE^-$	47
	hxA^- $lysE^+$	106
recombinants:	hxA^+ $lysE^+$	23
	hxA^- $lysE^-$	6

$$RF = \frac{23 + 6}{182} = 0.16$$

- ***hxA* and *riboD***

parentals:	hxA^+ $riboD^+$	45
	hxA^- $riboD^-$	77
recombinants:	hxA^+ $riboD^-$	25
	hxA^- $riboD^+$	32

$$RF = \frac{25 + 32}{182} = 0.31$$

taking into account double recombinants (some of which can only be scored if $lysE^+$. To correct for this it has been assumed that each unscorable class would have equal numbers to the corresponding class that could be scored.)

parentals:	$hxA^+ \cdot lysE^-$ $prtA^+$ $riboD^+$	not scorable
	hxA^- $lysE^+$ $prtA^-$ $riboD^-$	74
recombinants:	hxA^+ $lysE^+$ $prtA^-$ $riboD^-$	8
	hxA^- $lysE^-$ $prtA^+$ $riboD^+$	not scorable
	hxA^+ $lysE^-$ $prtA^-$ $riboD^-$	not scorable
	hxA^- $lysE^+$ $prtA^+$ $riboD^+$	7
	hxA^+ $lysE^-$ $prtA^+$ $riboD^-$	not scorable
	hxA^- $lysE^+$ $prtA^-$ $riboD^+$	22
double recombinants:	hxA^+ $lysE^+$ $prtA^+$ $riboD^+$	6
	hxA^- $lysE^-$ $prtA^-$ $riboD^-$	not scorable
	hxA^+ $lysE^+$ $prtA^-$ $riboD^+$	9
	hxA^- $lysE^-$ $prtA^+$ $riboD^-$	not scorable
	hxA^+ $lysE^-$ $prtA^-$ $riboD^+$	not scorable
	hxA^- $lysE^+$ $prtA^+$ $riboD^-$	3

$$RF = \frac{8 + 7 + 22 + 12 + 18 + 6}{129} = 0.57$$

- ***lysE* and *riboD***

parentals:	$lysE^+$ $riboD^-$	85
	$lysE^-$ $riboD^+$	33
recombinants:	$lysE^+$ $riboD^+$	45
	$lysE^-$ $riboD^-$	19

$$RF = \frac{45 + 19}{182} = 0.35$$

taking into account double recombinants (some of which can only be scored if *lysE*$^+$. As only 1 class from each category could be scored, these results were not corrected)

parentals:	*lysE*$^+$ *prtA*$^-$ *riboD*$^-$	85
	lysE$^-$ *prtA*$^+$ *riboD*$^+$	not scorable
single recombinants:	*lysE*$^+$·*prtA*$^+$ *riboD*$^+$	14
	lysE$^-$ *prtA*$^-$ *riboD*$^-$	not scorable
	lysE$^+$ *prtA*$^-$ *riboD*$^+$	31
	lysE$^-$·*prtA*$^+$ *riboD*$^-$	not scorable
double recombinants	*lysE*$^+$ *prtA*$^+$ *riboD*$^-$	6
	lysE$^-$·*prtA*$^-$ *riboD*$^+$	not scorable

$$RF = \frac{14 + 31 + 12}{136} = 0.42$$

Appendix 8.

Comparison of the levels of proteolytic activity produced by the wildtype strain MH97, *sB1* strain MK130, the *xprI1* strain MK169, and the *sB1 xprI1* strain, MK170, under sulphur-repressing and derepressing conditions.

Table A9.1. Raw data. Strains were grown for 20 hours in sulphur-repressing (sulphur = 1% glucose, 10 mM ammonium tartrate, 0.1% sodium thiosulphate) or sulphur-derepressing (no sulphur = 1% glucose, 10 mM ammonium tartrate) media. All minimal media used in this experiment were made using low-sulphur salt solution. Assays were carried out in triplicate, with internal duplicates, therefore assay number refers to assay: duplicate. Protease levels are in arbitrary units/gram dry weight mycelia.

strain	media	Assay number						mean	SD
		1.1	1.2	2.1	2.2	3.1	3.2		
MH97	sulphur	2.24	5.89	12.25	6.32	6.71	0.65	5.67	4.05
	no sulphur	43.02	39.02	260.40	375.70	77.67	41.64	139.58	131.16
MK130	sulphur	37.34	39.33	333.13	308.13	85.09	10.42	150.91	134.13
	no sulphur	110.65	84.63	437.14	372.80	137.50	125.77	211.42	152.33
MK169	sulphur	2.33	5.93	24.50	3.11	5.15	8.36	8.23	8.25
	no sulphur	4.92	6.58	18.75	21.60	4.35	10.17	11.06	7.40
MK170	sulphur	12.00	23.40	11.14	6.50	7.59	10.20	11.81	6.06
	no sulphur	23.40	23.10	16.94	21.60	9.22	2.32	16.10	8.61

- **Comparison between strains under sulphur-repressing conditions.**

ANOVA: single factor, MH97 compared to MK130.

SUMMARY

Groups	Count	Sum	Average	Variance
MH97	6	34.06	5.68	16.36
MK130	6	905.44	150.91	17990.81

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	63275.26	1	63275.26	**7.03**	0.02	**4.97**
Within groups	90035.86	10	9003.586			
Total	153311.10	11				

The F value of 7.03 is greater than the critical F value of 4.97, which indicates that the levels of protease activity produced by MK130 and MH97, under sulphur-repressing conditions as assayed at pH 7.2, are significantly different.

ANOVA: single factor, MH97 compared to MK169.

SUMMARY

Groups	Count	Sum	Average	Variance
MH97	6	34.06	5.68	16.36
MK169	6	49.38	8.23	68.11

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	19.56	1	19.56	**0.46**	0.51	**4.97**
Within groups	422.34	10	42.23			
Total	441.90	11				

The F value of 0.46 is less than the critical F value of 4.97, which indicates that the levels of protease activity produced by MK169 and MH97, under sulphur-repressing conditions as assayed at pH 7.2, are not significantly different.

ANOVA: single factor, MH97 compared to MK170.

SUMMARY

Groups	Count	Sum	Average	Variance
MH97	6	34.06	5.68	16.36
MK170	6	70.83	11.81	36.68

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	112.67	1	112.67	**4.25**	0.07	**4.97**
Within groups	265.22	10	26.52			
Total	377.89	11				

The F value of 4.25 is less than the critical F value of 4.97, which indicates that the levels of protease activity produced by MK170 and MH97, under sulphur-repressing conditions as assayed at pH 7.2, are not significantly different.

ANOVA: single factor, MK130 compared to MK169.

SUMMARY

Groups	Count	Sum	Average	Variance
MH130	6	905.44	150.91	17990.81
MK169	6	49.38	8.23	68.11

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	61069.89	1	61069.89	**6.76**	0.03	**4.97**
Within groups	90294.58	10	9029.46			
Total	151364.50	11				

The F value of 6.76 is greater than the critical F value of 4.97, which indicates that the levels of protease activity produced by MK130 and MH169, under sulphur-repressing conditions as assayed at pH 7.2, are significantly different.

ANOVA: single factor, MK130 compared to MK170.

SUMMARY

Groups	Count	Sum	Average	Variance
MH130	6	905.44	150.91	17990.81
MK170	6	70.83	11.81	36.68

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	58047.82	1	58047.82	**6.44**	0.03	**4.97**
Within groups	90137.46	10	9013.75			
Total	148185.30	11				

The F value of 6.44 is greater than the critical F value of 4.97, which indicates that the levels of protease activity produced by MK130 and MH170, under sulphur-repressing conditions as assayed at pH 7.2, are significantly different.

ANOVA: single factor, MK169 compared to MK170.

SUMMARY

Groups	Count	Sum	Average	Variance
MH169	6	49.38	8.23	68.11
MK170	6	70.83	11.81	36.68

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	38.34	1	38.34	**0.73**	0.41	**4.97**
Within groups	523.94	10	52.39			
Total	562.28	11				

The F value of 0.73 is less than the critical F value of 4.97, which indicates that the levels of protease activity produced by MK169 and MH170, under sulphur-repressing conditions as assayed at pH 7.2, are not significantly different.

- **Comparison between strains under sulphur-derepressing conditions.**

ANOVA: single factor, MH97 compared to MK130.

SUMMARY

Groups	Count	Sum	Average	Variance
MH97	6	837.45	139.58	20642.27
MK130	6	1268.49	211.415	23204.68

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	15482.96	1	15482.96	**0.71**	0.42	**4.97**
Within groups	219234.80	10	21923.48			
Total	234717.7	11				

The F value of 0.71 is greater than the critical F value of 4.97, which indicates that the levels of protease activity produced by MK130 and MH97, under sulphur-derepressing conditions as assayed at pH 7.2, are not significantly different.

ANOVA: single factor, MH97 compared to MK169.

SUMMARY

Groups	Count	Sum	Average	Variance
MH97	6	837.45	139.58	20642.27
MK169	6	66.37	11.06	54.76

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	49547.03	1	49547.03	**4.79**	0.05	**4.97**
Within groups	103485.20	10	10348.52			
Total	153032.20	11				

The F value of 4.79 is less than the critical F value of 4.97, which indicates that the levels of protease activity produced by MK169 and MH97, under sulphur-derepressing conditions as assayed at pH 7.2, are not significantly different.

ANOVA: single factor, MH97 compared to MK170.

SUMMARY

Groups	Count	Sum	Average	Variance
MH97	6	837.45	139.58	20642.27
MK170	6	96.58	16.10	74.09

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	45740.70	1	45740.70	**4.42**	0.07	**4.97**
Within groups	103581.80	10	10358.18			
Total	149322.5	11				

The F value of 4.42 is less than the critical F value of 4.97, which indicates that the levels of protease activity produced by MK170 and MH97, under sulphur-derepressing conditions as assayed at pH 7.2, are not significantly different.

ANOVA: single factor, MK130 compared to MK169.

SUMMARY

Groups	Count	Sum	Average	Variance
MK130	6	1268.49	211.42	23204.68
MK169	6	66.37	11.06	54.76

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	120424.40	1	120424.40	**10.36**	0.01	**4.97**
Within groups	116297.20	10	11629.72			
Total	236721.60	11				

The F value of 10.36 is greater than the critical F value of 4.97, which indicates that the levels of protease activity produced by MK130 and MH169, under sulphur-derepressing conditions as assayed at pH 7.2, are significantly different.

ANOVA: single factor, MK130 compared to MK170.

SUMMARY

Groups	Count	Sum	Average	Variance
MH130	6	1268.49	211.42	23204.68
MK170	6	96.58	16.10	74.09

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	114447.80	1	114447.80	**9.83**	0.01	**4.97**
Within groups	116393.90	10	11639.39			
Total	230841.60	11				

The F value of 9.83 is greater than the critical F value of 4.97, which indicates that the levels of protease activity produced by MK130 and MH170, under sulphur-repressing conditions as assayed at pH 7.2, are significantly different.

ANOVA: single factor, MK169 compared to MK170.

SUMMARY

Groups	Count	Sum	Average	Variance
MH169	6	66.37	11.06	54.76
MK170	6	96.58	16.10	74.09

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	76.05	1	76.05	**1.18**	0.30	**4.97**
Within groups	644.28	10	64.43			
Total	720.34	11				

The F value of 1.18 is less than the critical F value of 4.97, which indicates that the levels of protease activity produced by MK169 and MH170, under sulphur-derepressing conditions as assayed at pH 7.2, are not significantly different.

Summary of Results.

Under sulphur-repressing conditions the wildtype strain, MH97, the *xprI*$_1$ strain, MK169, and the *sB*$_1$ *xprI*$_1$ strain, MK170, do not produce significantly different level of protease activity. The *sB*$_1$ strain, MK130, produces significantly higher level of protease activity than any of the other strains examined under sulphur-repressing conditions. The high level of protease activity produced by MK130 under sulphur-repressing conditions is due to the *sB*$_1$ mutation. As seen in the *sB*$_1$ *xprI*$_1$ strain, MK170, the *xprI*$_1$ mutation suppresses this response.

Under sulphur-derepressing conditions the levels of protease activity produced by the wildtype strain, MH97, and the *sB*$_1$ strain, MK130, are not significantly different. The statistical analysis indicates that the level of protease activity produced by the strains carrying the *xprI*$_1$ mutation are not statistically different than the levels produced by the wildtype strains. The mean levels of activity produced by these two groups are quite different. The *xprI*$_1$ strains produced consistently lower levels of protease activity than wildtype strains. However, the variability in the level of protease activity produced by the wildtype strain resulted in a high standard deviation, and consequently the statistical analysis found no significant differences between the wildtype and *xprI*$_1$ strain. The *xprI*$_1$ mutation suppresses the effect of the *sB*$_1$ mutation on protease production.

Appendix 9.

Comparison of the levels of proteolytic activity produced by the wildtype strain MH97, *sB1* strain MK130, and the *xprI1* strain MK169, under nitrogen-repressing and derepressing conditions.

Table A9.1. Raw data. Strains were grown for 24 hours in minimal media containing 1% glucose as a carbon source and 10 mM ammonium tartrate as a nitrogen source, then transferred to nitrogen-repressing (nitrogen = 1% glucose, 10 mM ammonium tartrate, 0.1% sodium thiosulphate) or nitrogen-derepressing (no nitrogen = 1% glucose, 0.1% sodium thiosulphate) conditions for 16 hours. Assays were carried out in triplicate, with internal duplicates, therefore assay number refers to assay: duplicate. Protease levels are in arbitrary units of activity / gram dry weight mycelia.

strain	media	Assay number						mean	SD
		1.1	1.2	2.1	2.2	3.1	3.2		
MH97	nitrogen	5.65	4.84	2.57	3.73	7.35	6.05	5.03	1.71
	no nitrogen	51.45	68.90	31.25	21.67	42.38	49.28	44.16	16.54
MK130	nitrogen	55.2	51.92	70.83	31.71	56.81	82.80	58.21	17.42
	no nitrogen	28.51	25.40	22.35	15.75	35.40	33.74	26.86	7.33
MK169	nitrogen	18.03	2.93	5.41	5.65	12.96	7.44	8.74	5.66
	no nitrogen	6.28	3.73	17.44	24.56	6.21	9.80	11.34	6.77

- **Comparison between strains under nitrogen-repressing conditions.**

ANOVA: single factor, MH97 compared to MK130.

SUMMARY

Groups	Count	Sum	Average	Variance
MH97	6	30.19	5.03	2.92
MK130	6	349.27	58.21	303.35

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	8484.34	1	8484.34	**55.40**	2.2×10^{-5}	**4.97**
Within groups	1531.35	10	153.14			
Total	10015.69	11				

The F value of 55.40 is greater than the critical F value of 4.97, which indicates that the levels of protease activity produced by MK130 and MH97, under nitrogen-repressing conditions as assayed at pH 7.2, are significantly different.

ANOVA: single factor, MH97 compared to MK169.

SUMMARY

Groups	Count	Sum	Average	Variance
MH97	6	30.19	5.03	2.92
MK169	6	52.42	8.74	32.04

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	41.18	1	41.18	**2.36**	0.16	**4.97**
Within groups	174.78	10	17.48			
Total	215.96	11				

The F value of 2.36 is less than the critical F value of 4.97, which indicates that the levels of protease activity produced by MK169 and MH97, under nitrogen-repressing conditions as assayed at pH 7.2, are not significantly different.

ANOVA: single factor, MK130 compared to MK169.

SUMMARY

Groups	Count	Sum	Average	Variance
MH97	6	349.27	58.21	303.35
MK130	6	52.42	8.74	32.04

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	7343.33	1	7343.33	**43.79**	5.95×10^{-5}	**4.97**
Within groups	1676.96	10	167.70			
Total	9020.29	11				

The F value of 43.79 is greater than the critical F value of 4.97, which indicates that the levels of protease activity produced by MK130 and MH97, under nitrogen-repressing conditions as assayed at pH 7.2, are significantly different.

- **Comparison between strains under nitrogen-derepressing conditions.**

ANOVA: single factor, MH97 compared to MK130.

SUMMARY

Groups	Count	Sum	Average	Variance
MH97	6	264.93	44.16	273.41
MK130	6	161.15	26.86	53.78

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	897.52	1	897.52	**5.49**	0.041	**4.97**
Within groups	1635.96	10	163.60			
Total	2533.48	11				

The F value of 5.49 is greater than the critical F value of 4.97, which indicates that the levels of protease activity produced by MK130 and MH97, under nitrogen-derepressing conditions as assayed at pH 7.2, are significantly different.

ANOVA: single factor, MH97 compared to MK169.

SUMMARY

Groups	Count	Sum	Average	Variance
MH97	6	264.93	44.16	273.41
MK169	6	68.02	11.34	64.84

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	3231.13	1	3231.13	**19.11**	0.001	**4.97**
Within groups	1691.25	10	169.13			
Total	4922.37	11				

The F value of 19.11 is greater than the critical F value of 4.97, which indicates that the levels of protease activity produced by MK169 and MH97, under nitrogen-derepressing conditions as assayed at pH 7.2, are significantly different.

ANOVA: single factor, MK130 compared to MK169.

SUMMARY

Groups	Count	Sum	Average	Variance
MK130	6	161.15	26.86	53.78
MK169	6	68.02	11.34	64.84

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	722.77	1	722.77	**12.19**	0.006	**4.97**
Within groups	593.08	10	59.31			
Total	1315.84	11				

The F value of 12.19 is greater than the critical F value of 4.97, which indicates that the level of protease activity produced by MK130 and MH97, under nitrogen-repressing conditions as assayed at pH 7.2, are significantly different.

SUMMARY OF RESULTS:

Under nitrogen-repressing conditions the wildtype strain, MH97, and the *xprI₁* strain, MK169, do not produce significantly different level of protease activity. The *sB₁* strain, MK130, produces significantly higher level of protease activity than either of the other strains examined under nitrogen-repressing conditions. The high level of protease activity produced by MK130 under nitrogen-repressing conditions is due to the *sB₁* mutation.

Both MK130 and MK169 produce significantly less protease than MH97, under nitrogen-derepressing conditions. The *sB₁* strain, MK130, is producing protease in response to both nitrogen and sulphur limitation, which results in lower levels of protease than starvation in response to either stimulus alone. The *xprI₁* strain, MK169, produces less protease than the wildtype strain because the *xprI₁* mutation is able to suppress the response to nitrogen derepression. The *xprI₁* strain, MK169, also produces less protease than the *sB₁* strain, MK130, which produces substantial levels of protease, even though the level is significantly less than that produced by the wildtype strain.

Appendix 10.

Comparison of the levels of proteolytic activity produced by the wildtype strain MH97, *sB1* strain MK130, and the *xprI1* strain MK169, under carbon-repressing and derepressing conditions.

Table A10.1. Raw data. Strains were grown for 24 hours in minimal media, then transferred to carbon-repressing (carbon = 1% glucose, 10 mM ammonium chloride, o.1% sodium thiosulphate) or carbon-derepressing (no carbon = 10 mM ammonium chloride, 0.1% sodium thiosulphate) conditions for 16 hours. Assays were carried out in triplicate, with internal duplicates, therefore assay number refers to assay: duplicate. Protease levels are in arbitrary units/gram dry weight mycelia.

strain	media	Assay number						mean	SD
		1.1	1.2	2.1	2.2	3.1	3.2		
MH97	carbon	5.92	2.20	6.25	5.50	2.73	3.47	4.35	1.76
	no carbon	48.03	42.14	47.20	67.69	74.25	71.96	58.55	14.27
MK130	carbon	27.87	18.11	6.71	6.95	18.43	24.84	17.15	8.83
	no carbon	42.13	64.93	96.26	112.21	100.28	86.45	83.71	25.82
MK169	carbon	6.71	11.11	13.34	4.37	10.99	7.99	9.09	3.31
	no carbon	10.32	8.93	11.03	11.40	9.12	12.25	10.51	2.51

- **Comparison between strains under carbon-repressing conditions.**

ANOVA: single factor, MH97 compared to MK130.

SUMMARY

Groups	Count	Sum	Average	Variance
MH97	6	26.07	4.35	3.08
MK130	6	102.91	17.16	77.93

ANOVA

Source of variation	SS	df	MS	**F**	P-value	**F crit**
Between groups	492.03	1	492.03	**12.15**	0.006	**4.97**
Within groups	405.07	10	40.51			
Total	897.10	11				

The F value of 12.15 is greater than the critical F value of 4.97, which indicates that the levels of protease activity produced by MK130 and MH97, under carbon-repressing conditions as assayed at pH 7.2, are significantly different.

ANOVA: single factor, MH97 compared to MK169.

SUMMARY

Groups	Count	Sum	Average	Variance
MH97	6	26.07	4.35	3.08
MK169	6	54.51	9.09	10.98

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	67.40	1	67.40	**9.58**	0.005	**4.97**
Within groups	70.32	10	7.03			
Total	137.73	11				

The F value of 9.58 is greater than the critical F value of 4.97, which indicates that the levels of protease activity produced by MK169 and MH97, under carbon-repressing conditions as assayed at pH 7.2, are significantly different.

ANOVA: single factor, MK130 compared to MK169.

SUMMARY

Groups	Count	Sum	Average	Variance
MK130	6	102.91	17.15	77.93
MK169	6	54.51	9.09	10.98

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	195.21	1	195.21	**4.39**	0.063	**4.97**
Within groups	444.55	10	44.46			
Total	639.77	11				

The F value of 4.39 is less than the critical F value of 4.97, which indicates that the levels of protease activity produced by MK130 and MH97, under carbon-repressing conditions as assayed at pH 7.2, are not significantly different.

- **Comparison between strains under carbon-derepressing conditions.**

ANOVA: single factor, MH97 compared to MK130.

SUMMARY

Groups	Count	Sum	Average	Variance
MH97	6	351.27	58.55	203.73
MK130	6	502.26	83.71	666.68

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	1899.83	1	1899.83	**4.37**	0.063	**4.97**
Within groups	4352.05	10	435.21			
Total	6251.88	11				

The F value of 4.37 is less than the critical F value of 4.97, which indicates that the levels of protease activity produced by MK130 and MH97, under carbon-derepressing conditions as assayed at pH 7.2, are not significantly different.

ANOVA: single factor, MH97 compared to MK169.

SUMMARY

Groups	Count	Sum	Average	Variance
MH97	6	351.27	58.55	203.73
MK169	6	63.05	10.51	1.71

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	6922.56	1	6922.56	**67.39**	9.38×10^{-6}	**4.97**
Within groups	1027.19	10	102.72			
Total	7949.76	11				

The F value of 67.39 is greater than the critical F value of 4.97, which indicates that the levels of protease activity produced by MK169 and MH97, under carbon-derepressing conditions as assayed at pH 7.2, are significantly different.

ANOVA: single factor, MK130 compared to MK169.

SUMMARY

Groups	Count	Sum	Average	Variance
MH130	6	502.26	83.71	666.68
MK169	6	63.05	10.51	1.71

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	16075.45	1	16075.45	**48.10**	4.02×10^{-5}	**4.97**
Within groups	3341.96	10	334.20			
Total	19417.42	11				

The F value of 48.10 is greater than the critical F value of 4.97, which indicates that the levels of protease activity produced by MK130 and MH97, under carbon-repressing conditions as assayed at pH 7.2, are significantly different.

Summary of Results.

Under carbon-repressing conditions, all three strains produce low levels of protease activity. Statistical analysis shows that the level of protease activity produced by the wildtype strain, MH97, is significantly different to the level of protease produced by the other two strains. A comparison of the levels of protease activity produced by the *sB1* strain, MK130, and the *xprI1* strain, MK169, has shown that under carbon-limiting conditions the level of protease activity produced by these strains is not significantly different.

Under carbon-limiting conditions the wildtype strain, MH97, and the *sB1* strain, MK130, do not produce significantly different levels of protease activity. Statistical analysis shows that the *xprI1* strain, MK169, produces significantly less protease activity than either other strain.

The levels of protease activity produced by the *xprI1* strain, MK169, and the *sB1* strain, MK130, were found to be significantly different from the wildtype strain, MH97, but not statistically different from each other. All three strains produced quite low levels of protease activity under carbon-repressing conditions, and the MK169 data set contains values which overlap the other two data sets.

Under carbon-limiting conditions the *xprI1* strain, MK169, produces significantly lower levels of protease activity than either other strain. This shows that the *xprI1* mutation represses the response to carbon limitation.

Appendix 11.

Assays examining phosphatase activity in the culture filtrate of strains grown in phosphate-limiting media.

Table A11.1. Assay results. Phosphatase activity is in arbitrary units of activity / gram dry weight mycelia. The acid and alkaline phosphatase levels produced by the wildtype strain MH97, the *xprI*$_1$ strain MK169, and the *xprE*$_1$ strain MK43 were assayed at pH 6.0 and pH 10.0. Cultures were grown for 16 hours in minimal media containing 1% glucose and 10 mM ammonium tartrate (made from phosphate containing salt solution) then transferred to phosphate-repressing (+P = 1% glucose, 10 mM ammonium tartrate, phosphate containing salt solution) or phosphate-derepressing (-P = 1% glucose, 10 mM ammonium tartrate, low phosphate salt solution) for 6 hours. All solutions used to make media for this experiment contained sulphate.

strain	conditions	assay pH	Assay number			mean	SD
			1	2	3		
MH97	+P	6	33.20	27.26	22.75	27.74	5.24
		10	29.06	24.61	17.58	25.74	5.79
	-P	6	265.29	155.25	211.11	210.55	55.02
		10	223.51	110.57	207.11	180.40	49.85
MK169	+P	6	37.92	48.33	42.44	42.90	5.21
		10	24.78	37.00	35.96	32.58	6.78
	-P	6	354.20	325.48	782.00	487.23	255.68
		10	259.13	280.18	373.48	304.26	60.86
MK43	+P	6	21.93	32.83	35.88	30.21	7.33
		10	17.39	32.83	15.92	22.05	9.37
	-P	6	195.29	339.13	172.94	235.79	90.19
		10	185.16	300.52	328.94	271.54	76.15

- **Comparison of activity at pH 6.0.**

ANOVA: single factor, comparison of MH97 and MK169 under phosphate-repressing conditions

SUMMARY

Groups	Count	Sum	Average	Variance
MH97	3	83.21	27.74	27.47
MK169	3	129.15	43.05	27.11

ANOVA

Source of variation	SS	df	MS	**F**	P-value	**F crit**
Between groups	351.75	1	351.75	**12.89**	0.23	**7.71**
Within groups	109.16	4	27.29			
Total	460.91	5				

The F value of 12.89 is greater than the critical F value of 7.71, which indicates that the levels of phosphatase activity produced by MK169 and MH97, under phosphate-repressing conditions as assayed at pH 6.0, are significantly different.

ANOVA: single factor, comparison of MH97 and MK43 under phosphate-repressing conditions

SUMMARY

Groups	Count	Sum	Average	Variance
MH97	3	83.21	27.74	27.47
MK43	3	90.64	30.21	53.79

ANOVA

Source of variation	SS	df	MS	**F**	P-value	**F crit**
Between groups	9.20	1	9.20	**0.23**	0.66	**7.71**
Within groups	162.51	4	40.63			
Total	171.72	5				

The F value of 0.23 is less than the critical F value of 7.71, which indicates that the levels of phosphatase activity produced by MH97 and MK43, under phosphate-repressing conditions as assayed at pH 6.0, are not significantly different.

ANOVA: single factor, comparison of MK169 and MK43 under phosphate-repressing conditions

SUMMARY

Groups	Count	Sum	Average	Variance
MK169	3	129.15	43.05	27.11
MK43	3	90.64	30.21	53.79

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	247.17	1	247.17	**6.11**	0.23	**7.71**
Within groups	161.79	4	40.45			
Total	408.96	5				

The F value of 6.11 is less than the critical F value of 7.71, which indicates that the levels of phosphatase activity produced by MK169 and MK43, under phosphate-repressing conditions as assayed at pH 6.0, are not significantly different.

ANOVA: single factor, comparison of MH97 and MK169 under phosphate-derepressing conditions

SUMMARY

Groups	Count	Sum	Average	Variance
MH97	3	631.65	210.55	3027.44
MK169	3	1461.68	487.23	65374.70

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	114825.00	1	114825.00	**3.36**	0.14	**7.71**
Within groups	136804.30	4	34201.07			
Total	251629.20	5				

The F value of 3.36 is less than the critical F value of 7.71, which indicates that the levels of phosphatase activity produced by MH97 and MK169, under phosphate-derepressing conditions as assayed at pH 6.0, are not significantly different.

ANOVA: single factor, comparison of MH97 and MK43 under phosphate-derepressing conditions

SUMMARY

Groups	Count	Sum	Average	Variance
MH97	3	631.65	210.55	3027.44
MK43	3	707.36	235.79	8134.76

ANOVA

Source of variation	SS	df	MS	**F**	P-value	**F crit**
Between groups	955.33	1	955.33	**0.17**	0.70	**7.71**
Within groups	22324.40	4	5581.10			
Total	23279.7	5				

The F value of 0.17 is less than the critical F value of 7.71, which indicates that the levels of phosphatase activity produced by MH97 and MK43, under phosphate-derepressing conditions as assayed at pH 6.0, are not significantly different.

ANOVA: single factor, comparison of MK169 and MK43 under phosphate-derepressing conditions

SUMMARY

Groups	Count	Sum	Average	Variance
MK169	3	1461.68	487.23	65374.70
MK43	3	707.36	235.79	8134.76

ANOVA

Source of variation	SS	df	MS	**F**	P-value	**F crit**
Between groups	94833.11	1	94833.11	**2.58**	0.18	**7.71**
Within groups	147018.90	4	36754.73			
Total	241852.00	5				

The F value of 2.58 is less than the critical F value of 7.71, which indicates that the levels of phosphatase activity produced by MH97 and MK43, under phosphate-derepressing conditions as assayed at pH 6.0, are not significantly different.

- **Comparison of activity at pH 10.0.**

ANOVA: single factor, comparison of MH97 and MK169 under phosphate-repressing conditions

SUMMARY

Groups	Count	Sum	Average	Variance
MH97	3	71.25	23.75	33.50
MK169	3	97.74	32.58	45.90

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	116.95	1	116.95	**2.95**	0.16	**7.71**
Within groups	158.81	4	9.70			
Total	275.76	5				

The F value of 2.95 is lower than the critical F value of 7.71, which indicates that the levels of phosphatase activity produced by MK169 and MH97, under phosphate-repressing conditions as assayed at pH 10.0, are not significantly different.

ANOVA: single factor, comparison of MH97 and MK43 under phosphate-repressing conditions

SUMMARY

Groups	Count	Sum	Average	Variance
MH97	3	71.25	23.75	33.50
MK43	3	66.14	22.05	87.75

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	4.35	1	4.35	**0.07**	0.80	**7.71**
Within groups	242.51	4	60.63			
Total	246.86	5				

The F value of 0.07 is less than the critical F value of 7.71, which indicates that the levels of phosphatase activity produced by MH97 and MK43, under phosphate-repressing conditions as assayed at pH 10.0, are not significantly different.

ANOVA: single factor, comparison of MK169 and MK43 under phosphate-repressing conditions

SUMMARY

Groups	Count	Sum	Average	Variance
MK169	3	97.74	32.58	45.90
MK43	3	66.14	22.05	87.75

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	166.43	1	166.43	**2.49**	0.19	**7.71**
Within groups	267.30	4	66.83			
Total	433.73	5				

The F value of 2.49 is less than the critical F value of 7.71, which indicates that the levels of phosphatase activity produced by MK169 and MK43, under phosphate-repressing conditions as assayed at pH 10.0, are not significantly different.

ANOVA: single factor, comparison of MH97 and MK169 under phosphate-derepressing conditions

SUMMARY

Groups	Count	Sum	Average	Variance
MH97	3	498.08	166.03	2484.94
MK169	3	912.79	304.26	3703.99

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	28664.06	1	28664.06	**9.26**	0.14	**7.71**
Within groups	12377.85	4	3094.46			
Total	41041.91	5				

The F value of 9.26 is greater than the critical F value of 7.71, which indicates that the levels of phosphatase activity produced by MH97 and MK169, under phosphate-derepressing conditions as assayed at pH 10.0, are significantly different.

ANOVA: single factor, comparison of MH97 and MK43 under phosphate-derepressing conditions

SUMMARY

Groups	Count	Sum	Average	Variance
MH97	3	498.08	166.03	2484.94
MK43	3	814.62	271.54	5798.05

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	166699.60	1	16699.60	**4.03**	0.12	**7.71**
Within groups	16565.98	4	4141.50			
Total	33265.57	5				

The F value of 4.03 is less than the critical F value of 7.71, which indicates that the levels of phosphatase activity produced by MH97 and MK43, under phosphate-derepressing conditions as assayed at pH 10.0, are not significantly different.

ANOVA: single factor, comparison of MK169 and MK43 under phosphate-derepressing conditions

SUMMARY

Groups	Count	Sum	Average	Variance
MK169	3	912.79	304.26	3703.99
MK43	3	8.14.62	271.54	5798.05

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	1606.23	1	1606.23	**0.34**	0.59	**7.71**
Within groups	19004.23	4	4751.02			
Total	20610.30	5				

The F value of 0.34 is less than the critical F value of 7.71, which indicates that the levels of phosphatase activity produced by MH97 and MK43, under phosphate-derepressing conditions as assayed at pH 6.0, are not significantly different.

SUMMARY OF RESULTS.

Phosphatase activity at pH 6.0, phosphate-repressing conditions:

Though the levels of phosphatase activity produced by MH97 and MK169 were significantly different, the level of phosphatase produced by MK43 was not significantly different from the levels produced by either MH97 or MK169.

Phosphatase activity at pH 6.0, phosphate-derepressing conditions:

Overall it appears that there is no significant difference between the strains under these conditions.

Phosphatase activity at pH 10.0, phosphate-repressing conditions:

Overall it appears that there is no significant difference between the strains under these conditions.

Phosphatase activity at pH 10.0, phosphate-derepressing conditions:

Though the levels of phosphatase activity produced by MH97 and MK169 were significantly different, the level of phosphatase produced by MK43 was not significantly different from the levels produced by either MH97 or MK169.

Appendix 12.

Results of the cross between strains carrying the *xprI*$_1$ and *xprG*$_2$ mutations.

MK199 = *pabA*$_1$; *xprG*$_2$

MK170 = *yA*$_1$ *suA-adE*$_{20}$ *adE*$_{20}$; *pyroA*$_4$; *nicB*$_8$; *xprI*$_1$ T2(II:VII)

Table A8.1. Raw data. The *xprG*$_2$ and *xprI*$_1$ mutations result in the same phenotype on plates where milk is the sole nitrogen source. Both mutations are linked to chromosome VII. The aim of this cross was to determine if these two mutations were alleles of the same gene. If they were allelic, it would be expected that all segregants would not produce protease on media where milk is the sole nitrogen source. If they were not allelic, segregants with a wildtype phenotype, able to produce protease when milk is the sole nitrogen source, would be expected. The proportion of wildtype progeny would be dependent on the linkage relationship between the two genes. Approximately 1/2 the segregants observed on spread plates showed aberrant morphology and were designated as "fluffy". The "fluffy" segregants produced many aerial hyphae and appeared white, though conidia could be observed when "fluffy" segregants were viewed under the dissecting microscope.

growth on complete media	halo production on media where milk is the sole nitrogen source	number of segregants
normal	no halo	46
normal	halo	18
"fluffy"	no halo	28
"fluffy"	halo	11
total		103

As wildtype segregants were observed, the *xprG*$_2$ and *xprI*$_1$ mutations must be in different genes.

Chi-square analysis.

If *xprG*$_2$ and *xprI*$_1$ are unlinked then it would be expected that 1/4 of the progeny would produce protease on media where milk is the sole nitrogen source, and 3/4 of the progeny would not.

phenotype	observed	expected
halo	29	25.75
no halo	74	77.25
total	103	103

$\chi^2 = (29-25.75)^2/25.75 + (74-77.25)^2/77.25 = 0.41 + 0.14 = 0.55$

d/f = 1

$\chi^2_{(P = 0.05)} = 3.84$

As the χ^2 result of 0.55 is less than the critical $\chi^2_{(P = 0.05)}$ of 3.84, the null hypothesis, which stated that if *xprG* and *xprI* were unlinked the progeny of this cross would conform to a ratio of 1:3 halo: no halo, is supported.

Appendix 13.
Crosses to strains carrying the *xprJ1* mutation.

- **results from the cross between strains carrying the *xprJ1* and *xprF1* mutations.**

Parental strains: PVK11 = *wA1; areA19; pyroA4; sB1; xprJ1*

MK117 = *yA1 pabA1 acuE215; xprF1*

Table A13.1. Results from the cross between strains carrying the *xprJ1* and *xprF1* mutations. Results for segregants carrying the *sB1* mutation only. Due to epistatic effects, the *yA* genotype cannot be determined in the *wA1* background, on the table this is marked n/s (not scorable).

marker							number of segregants
yA	*wA*	*pabA*	*pyroA*	*sB*	*xprF*	*xprJ*	
+	+	+	+	-	+	+	7
n/s	-	+	+	-	-	+	4
n/s	-	-	+	-	-	+	4
n/s	-	+	-	-	+	+	3
-	+	-	+	-	-	+	3
n/s	-	+	+	-	+	+	3
-	+	-	-	-	+	+	2
n/s	-	-	+	-	+	-	2
+	+	+	+	-	-	+	2
n/s	-	-	+	-	+	+	2
-	+	-	+	-	+	+	2
+	+	+	-	-	+	+	1
n/s	-	+	-	-	-	+	1
n/s	-	-	+	-	-	-	1 *
+	+	-	-	-	-	+	1
+	+	-	+	-	-	+	1
-	+	+	+	-	+	+	1
n/s	-	-	-	-	+	+	1
-	+	-	+	-	-	-	1 *
-	+	-	-	-	-	+	1
-	+	-	-	-	+	-	1
Total							40

* Two $xprF_1$ $xprJ_1$ segregants were identified on the basis of growth on hypoxanthine as the sole nitrogen source ($xprF_1$ strains are red in colour when grown on this media), and protease production on media where skim milk was the sole sulphur source ($xprJ_1$ strains do not produce extracellular protease). These $xprF_1$ $xprJ_1$ segregants produced extracellular protease on media where milk is the sole nitrogen source.

- **results from the cross between strains carrying the $xprJ_1$ and $xprG_1$ mutations.**

Parental strains:

PVK11 = wA_1; $areA_{19}$; $pyroA_4$; sB_1; $xprJ_1$

MK86 = yA_1 suA-adE_{20} adE_{20}; $xprG_1$; $niiA4$; $riboB_2$

Table A13.2. Results from the cross between strains carrying the $xprJ_1$ and $xprG_1$ mutations. Results for segregants carrying the sB_1 mutation only. Due to epistatic effects, the *yA* genotype cannot be determined in the wA_1 background, on the table this is marked n/s (not scorable).

marker							number of
yA	***wA***	***pyroA***	***riboB***	***sB***	***xprG***	***xprJ***	**segregants**
n/s	-	+	-	-	+	+	7
n/s	-	-	-	-	+	+	4
n/s	-	+	+	-	+	+	3
n/s	-	-	-	-	+	-	3
-	+	-	+	-	+	+	2
+	+	-	-	-	+	+	2
-	+	+	-	-	+	+	2
n/s	-	-	+	-	+	+	1
+	+	+	+	-	+	+	1
+	+	+	-	-	+	+	1
-	+	+	+	-	+	+	1
+	+	+	+	-	-	+	1
-	+	-	-	-	+	+	1
n/s	-	+	+	-	-	+	1
n/s	-	+	-	-	-	+	1
n/s	-	-	-	-	-	-	1
n/s	-	-	-	-	-	+	1 *
total							33

* The segregant scored as *xprG*$_1$ *xprJ*$_1$ was scored *xprG*$_1$ on media containing hypoxanthine as the sole nitrogen source, where it was a reddish coloured colony (typical of *xprG*$_1$ strains grown on this media), the colony did not produce protease on media where milk was the sole nitrogen or sulphur source. This segregant did not appear to carry the *areA*$_{19}$ mutation (which is also segregating in this cross) as it grew reasonably well on hypoxanthine as the sole nitrogen source, therefore it appears that *xprJ*$_1$ suppresses the *xprG*$_1$ phenotype on media where milk is the sole nitrogen source.